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## Meeting Report

**UKEMS / Dutch EMS-sponsored workshop on biomarkers of exposure and oxidative DNA damage & 7<sup>th</sup> GUM-<sup>32</sup>P-postlabelling workshop, University of Münster, Münster, Germany, 28-29 March 2011†**

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†This report is dedicated to Prof. Elmar Richter (Walther Straub Institute, Department of Toxicology, Ludwig-Maximilians-University, Munich, Germany), on the occasion of his retirement. In 1994 he organised the first <sup>32</sup>P-Postlabelling workshop in Munich.

**Speakers:** Volker M.Arlt (Institute of Cancer Research, UK), Frederick A.Beland (National Center for Toxicological Research; USA), Karen Brown (University of Leicester, UK), Marcus Cooke (University of Leicester, UK), Andrew Collins (University of Oslo, Norway), Silvio De Flora (University of Genoa, Italy), Eugenia Dogliotti (Istituto Superiore di Sanità, Italy), Bernd Epe (University of Mainz, Germany), Peter B.Farmer (University of Leicester, UK), Hansruedi Glatt (German Institute of Human Nutrition, Germany), Roger W.Godschalk (University of Maastricht, The Netherlands), Stephen S.Hecht (University of Minnesota, USA), Hanna L.Karlsson (Karolinska Institute, Sweden), Micheline Kirsch-Volders (Vrije Universiteit Brussels, Belgium), Soterios Kyrtopoulos (National Hellenic Research Foundation, Greece), Steffen Loft (University of Copenhagen, Denmark), Ryszard Olinski (Nicolaus Copernicus University, Poland), David H.Phillips (Institute of Cancer Research, UK), Miriam C.Poirier (National Cancer Institute, USA), Roel Schins (University of Düsseldorf, Germany), Heinz H.Schmeiser (German Cancer Research Center, Germany), Tanja Schwerdtle (University of Münster, Germany), Albrecht Seidel (Biochemical Institute for Environmental Carcinogens, Germany), Günter Speit (University of Ulm, Germany), Marie Stiborova (Charles University Prague, Czech Republic), Helga Stopper (University of Würzburg, Germany), Jan Topinka (Institute of Experimental Medicine AS, Czech Republic), Frederik-Jan van Schooten (University of Maastricht, The Netherlands), Roel Vermeulen (Utrecht University, The Netherlands).

Environmental exposures are a major concern for human cancer. However, the precise contribution of specific risk factors and their interactions, both with each other and with genotype, continue to be difficult to elucidate. The exposome is the comprehensive characterisation of an individual's lifetime exposure history (Wild [2009] *Mutagenesis* 24: 117-125). Unravelling complex environmental and genetic aetiologies in order to plan effective public health interventions demands that both environmental exposures and genetic variations are reliably measured. The development, validation and application of biomarkers of exposure are manifestly critical to the future of cancer epidemiology. The aim of this workshop at the University of Münster was to discuss the current status of exposure biomarkers in cancer molecular epidemiology as well as new findings achieved by applying the methods to studies of mechanisms of human cancer. Day one focused on biomarkers of exposure (*i.e.* carcinogen DNA adducts), effect and susceptibility to gain greater understanding of environmental cancer risks and their modulation. Day two focused on the role of oxidative stress and DNA damage in human carcinogenesis including methodologies

used for the measurement of oxidatively induced DNA lesions in human cells or tissues and the possible use of these lesions as cancer biomarkers.

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The United Kingdom Environmental Mutagen Society (UKEMS), the Dutch Environmental Mutagen Society (Dutch EMS) and the German Environmental Mutagen Society (GUM) sponsored a two-day workshop (28-29 March 2011) at the University of Münster in Münster, Germany, on biomarkers of exposure and oxidative damage to DNA to gain a greater understanding of environmental cancer risks and their modulation [1,2]. After nearly 5 years this is the 2<sup>nd</sup> international workshop on this topic after the first one was held September 2006 in Heidelberg, Germany, jointly sponsored by the Environmental Cancer Risk, Nutrition and Individual Susceptibility (ECNIS) EU Network of Excellence and the GUM [3]. The workshop in Münster provided a unique forum bringing together scientists working in this research area from Europe, United States, South America and Asia. More than 180 researchers from 20 countries took part. Within the workshop 29 lectures were given and nearly 80 posters presented.

The opening keynote lecture entitled ‘Carcinogen biomarkers for investigating tobacco and cancer’ was given by Stephen Hecht (University of Minnesota, USA). Among lifestyle factors definitely related to cancer, tobacco use arguably entails the largest human exposure to diverse chemical carcinogens including tobacco-specific nitrosamines (*e.g.* 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone [NNK], 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol [NNAL]), aldehydes, and polycyclic aromatic hydrocarbons (PAHs) [4]. People exposed to secondhand tobacco smoke (SHS) inhale the lung carcinogen NNK which is metabolised to NNAL which can be measured in urine and has emerged as an excellent biomarker of NNK uptake. Urine samples from 79 children exposed to SHS were analysed for total NNAL [5]. The study showed that children were nearly all exposed to NNK due mainly to exposure to SHS from adult smokers in their homes implicating that adult smokers should adopt restrictions to protect their children from SHS. Another validated biomarker of PAH exposure is *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT) and urinary levels of PheT and NNAL in relation to lung cancer development in cigarette smokers were examined in the Shanghai Cohort Study [6]. This case-control study nested within the cohort included 475 lung cancer patients (cases) who smoked cigarettes at recruitment and 475 current smokers (controls). Urinary levels of PheT and NNAL were significantly higher for cases than controls after adjustment for smoking intensity and duration confirming them as risk biomarkers for lung cancer in smokers. Albrecht Seidel (Biochemical Institute for

Environmental Carcinogens, Germany) reported that the determination of urinary excreted PAH metabolites (*e.g.* of pyrene and phenanthrene) can be used in human biomonitoring at the workplace [7,8]. The occupational settings included employees of a manufacturer of graphite electrodes, employees of a coking plant and employees feeding converters during steel production. Overall, phenanthrene diols appeared to be sensitive biomarkers of PAH exposure. The various types of phenanthrene metabolites (phenols, diols and phenanthrene tetrol) could probably be used in future studies for phenotyping of individuals to determine their possible susceptibility to develop cancer upon exposure to PAH mixtures.

Silvio De Flora (University of Genoa, Italy) delivered a talk on ‘Induction of biomarker and tumours by cigarette smoke (CS), and their chemoprevention’. Chemopreventive agents of natural or pharmacological origin, such as *N*-acetyl-L-cysteine (NAC), phenethyl isothiocyanate (PEITC), and budesonide, have been shown to modulate CS-altered end-points (*e.g.* DNA adduct formation, oxidative damage to DNA, gene expression) [9]. However, only recently a suitable animal model for evaluating CS carcinogenicity was developed. This mouse model was successfully used to demonstrate the ability of NAC, PEITC and budesonide to prevent smoke-induced lung cancer, according to protocols mimicking the situation either in current smokers or in ex-smokers [10]. NAC and ascorbic acid were also successful to prevent lung cancer induced by mainstream CS after birth when they were administered during prenatal life. Transplacental CS carcinogenicity and its modulation were also investigated [11]. Miriam Poirier (National Cancer Institute, USA) evaluated the chemopreventive potential of chlorophyllin (CHL) [12] in a mouse model of esophageal cancer in cancer-susceptible mice (*i.e.* *Xpa*<sup>-/-</sup>, *Tp53*<sup>+/-</sup> mice) fed with benzo[*a*]pyrene (BaP) in the diet. Exceedingly high esophageal cancer rates in Linxian, China, have been associated with the ingestion and inhalation of PAHs in the ambient environment [13]. Wild-type (WT) mice, which are repair proficient, fed with 100 ppm BaP typically had less DNA damage than repair-deficient *Xpa*(<sup>-/-</sup>)*Tp53*(<sup>+/-</sup>) mice in esophagi, livers and lungs. Mice fed with 100 ppm BaP/0.3% CHL had reduced BaP-DNA adduct levels in the liver, but no change in other organs, compared to mice fed with BaP alone, indicating that, contrary to expectations, CHL did not always decrease BaP-induced DNA damage *in vivo*. ‘What BaP does to cells, and what cells do to BaP’ was discussed by David Phillips (Institute of Cancer Research, UK). Discovered and identified more than 75 years ago, the environmental carcinogen BaP is still widely studied and has become a standard test agent for exploring the metabolic capacity of biological systems and the responses of cells or tissues *in vitro* or *in vivo* to external genotoxic insult. Recent studies demonstrated that cytochrome

P450s (CYPs), involved in BaP metabolism, seems to be more important for detoxication of BaP *in vivo* despite being important for its bioactivation *in vitro* [14]. DNA adduct formation by BaP, but not by its reactive metabolite BaP-diolepoxide (BPDE), appears to be p53-dependent, suggesting that loss of p53 affects metabolic activation [15]. Meanwhile *in vivo* studies have shown that BaP forms DNA adducts with equal measure in both target and non-target tissues, while gene expression changes are organ specific.

Frederik-Jan van Schooten (University of Maastricht, The Netherlands) discussed that low oxygen concentrations in chronic obstructive pulmonary disease (COPD) [16] affected lung tissues may be involved in the enhanced lung cancer risk. He showed that hypoxia-related activation of hypoxia-inducible factor 1 (HIF1 $\alpha$ ) amplifies a BaP-induced mutagenic phenotype [17]. Several processes may in part be responsible for this phenotype: (i) changes in BaP metabolism; (ii) transmembrane transport of BaP or its metabolites; and (iii) the repair capacity of the subsequent DNA damage. For example, cells in which HIF1 $\alpha$  is dysregulated (VHL mutants) had an approximately 10-fold decrease in the ability to repair BaP-DNA adducts. Enhanced cancer risks in COPD patients may be a result of inflammatory-related DNA damaging effects and oxygen restriction leading to hypoxia. Micheline Kirsch-Volders (Vrije Universiteit Brussels, Belgium) reported that birth can be seen as a ‘hyperoxic’ challenge since newborns leave an intra-uterine ‘hypoxic’ environment to enter a ‘normoxic’ environment. The *in vitro* genetic susceptibility for hydrogen peroxide in peripheral blood mononuclear cells (PBMC) of mother-fullterm newborn and mother-preterm newborn pairs was investigated using the comet and micronucleus (MN) [18] assay, taking into account some relevant genotypes, *e.g.* in DNA repair [19,20]. Overall, fullterm newborns were less sensitive to the *in vitro* induced oxidative stress as compared to adults. Mothers accumulated higher background frequencies of micronuclei as compared to their fullterm newborn daughters, confirming the age factor. Newborns carrying the variant *XRCC3*<sup>241</sup> genotype were at higher risk of MN by oxidative stress. Günter Speit (University of Ulm) critically discussed ‘The MN test as a biomarker for cancer risk and exposure to genotoxic agents’ [21]. The major questions addressed were: What is the origin of micronuclei? Is the MN frequency a predictor of cancer risk? Is the cytokinesis-block micronucleus test (CBMNT) a sensitive test for human biomonitoring of mutagen-exposed populations? Conclusions included that the CBMNT is designed to detect micronuclei in lymphocytes after division *in vitro*. Micronuclei *in vivo* will not significantly contribute to the micronuclei frequency in binuclear cells. Cancer risks are associated with higher baseline MN frequencies. It has not been shown that cancer risks are associated with environmental exposure to mutagens. MN can be formed before

cytochalasin-B is added and these cells will usually not appear as binucleated cells with micronuclei. The standard protocol of the CBMNT used in biomonitoring is not sensitive for the detection of cells damaged *in vivo*.

Soterios Kyrtopoulos delivered a talk on ‘Progress in the use of traditional and omics-based biomarkers of exposure in population studies’. Despite extensive development of different technologies of biomarkers of carcinogenic exposure, in view of the growing need for larger population-based studies assay sensitivity and throughput have become limiting factors. Progress has been made in developing sensitive ELISA-type immunoassays to detect DNA adducts of environmental interest such as O<sup>6</sup>-methylguanine and BPDE-type adducts. The assay uses anti-adduct antibodies to bind selectively to a solid surface adduct-containing DNA fragments, followed by quantitation of the latter with anti-DNA antisera [22]. Assays have been used for the analysis of more than 1,000 DNA samples from maternal and cord blood in the context of the NewGeneris project [23] confirming their applicability in population studies. Assays can be extended to other DNA adducts, and have the potential for automation and multiplexing.

Several presentations focused on the <sup>32</sup>P-postlabelling method to detect DNA adducts. Heinz Schmeiser (German Cancer Research Center, Germany) discussed ‘DNA adducts formed by arachidonic acid metabolites’. Arachidonic acid derived mediators, the eicosanoids, play a fundamental role in physiological and pathological processes involved in inflammatory responses. Reactive metabolites include LTA<sub>4</sub> (5,6-epoxy-7,9,11,14-eicosatetraenoic acid) and four *cis*-epoxyeicosatrienoic acids (14,15-, 11,12-, 8,9- and 5,6-EET). When the human monocytic cell line Mono Mac 6 was stimulated with arachidonic acid and calcium ionophore LTA<sub>4</sub>-DNA adducts were detected by <sup>32</sup>P-postlabelling, the major ones being derived from deoxyguanosine [24]. EETs were also capable of forming adducts, primarily with deoxyguanosine; highest DNA binding was found with 11,12-EET. The method seems to be suitable to study the relationship between inflammation and the onset of cancer. Jan Topinka (Institute of Experimental Medicine AS CR, Czech Republic) evaluated the genotoxicity of complex mixtures of air pollutants (*e.g.* PAHs and their derivatives) bound to PM<sub>2.5</sub> ambient air dust particles collected in various localities of the Czech Republic which differed in the extent and sources of air pollution [25]. Organic extracts were prepared and DNA adduct formation was assessed by <sup>32</sup>P-postlabelling and related to the carcinogenic PAHs (c-PAHs) content. The study confirmed that c-PAHs and particularly BaP are mostly responsible for the genotoxicity of PM<sub>2.5</sub>. Gene expression analysis in mammalian cells revealed that multiple transcripts/genes were significantly correlated with BaP content in organic extracts and DNA



adduct levels. Marie Stiborova (Charles University Prague, Czech Republic) reported on the 'Detection of DNA adducts formed by environmental pollutants by  $^{32}\text{P}$ -postlabelling' including the azo dye 1-phenylazo-2-naphthol (Sudan I) [26], the nitro-aromatics 3-nitrobenzanthrone (3-NBA) and 2-nitroanisole, and aromatic amines such as *o*-anisidine [27]. Recent studies on the air pollutant 3-NBA showed that it induces the cytosolic nitroreductase NAD(P)H:quinone oxidoreductase (NQO1) in liver, kidney and lungs of rats treated by intra-tracheal instillation, thereby enhancing its own genotoxic and carcinogenic potential [28]. In contrast, the 3-NBA isomer 2-nitrobenzanthrone (2-NBA) was not metabolised by NQO1; no 2-NBA-derived DNA adducts were detectable [29]. Molecular docking of 2-NBA and 3-NBA to the active site of NQO1 showed similar binding affinities; however, the binding orientation of 2-NBA does not favour the reduction of the nitro group. These results suggest that 2-NBA possesses a lower risk to humans than 3-NBA. The role of CYP enzymes in the detoxication and activation of aristolochic acid (AA) was discussed by Volker Arlt (Institute of Cancer Research, UK). AAI, the main ingredient of *Aristolochia* species, represents one of the few carcinogens of which the relationship between DNA adduct formation and human cancer has been defined [30]. The predominant DNA adduct formed *in vivo* is 7-(deoxyadenosin- $\text{N}^6$ -yl)aristolactam I, which is a premutagenic lesion leading to AT to TA transversions in the *TP53* gene [31]. The metabolism of AAI was investigated using the HRN (Hepatic Cytochrome P450 Reductase Null) mouse model in which the unique electron donor to CYPs is deleted specifically in the liver, resulting in the loss of essentially all hepatic CYP function [32]. Hepatic CYPs demethylated AAI *in vitro* to the detoxication metabolite 8-hydroxyaristolochic acid I (aristolochic acid Ia), indicating that less AAI is distributed to extra-hepatic organs in WT mice. Indeed, AAI-DNA adduct levels (measured by  $^{32}\text{P}$ -postlabelling) in organs of HRN mice, having low hepatic AAI demethylation capacity, were increased significantly. AAI demethylation was attributable mainly to mouse CYP1A1 and CYP1A2, indicating that AAI activation and detoxication by CYPs in mice are mainly dictated by its binding affinity to CYP1A1 and CYP1A2, by their turnover and by the balance between the ability of CYP1A enzymes to oxidize and reduce AAI.

Frederick Beland (National Center for Toxicological Research, USA) and Hansruedi Glatt (German Institute of Human Nutrition, Germany) both discussed the genotoxic risk from dietary exposure. Beland reported that acrylamide [33,34] and furan have been detected as contaminants in many common foods cooked at high temperatures, and are carcinogenic in laboratory animals. Two-year chronic bioassays were conducted in F344 rats with acrylamide and furan to provide dose-response data for tumour induction. In addition, mechanistic studies

were performed to characterise the conversion of acrylamide to glycidamide and furan to *cis*-butene-1,4-diol in the carcinogenic process. Collectively, the data show that acrylamide is carcinogenic through a mechanism involving the formation of glycidamide; furan is also carcinogenic but the mechanism at the present time is uncertain. Glatt reported on 'DNA adducts by food constituents in mouse models genetically modified in the sulfotransferase (SULT) status'. *In vitro* and *in vivo* models were developed for studying SULT-dependent genotoxigants. DNA adduct formation in *SULT1A1/1A2*-humanised mouse tissues was enhanced by compounds including 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and 1-hydroxymethylpyrene. The influence of the *SULT1A1/1A2* transgene on adduct formation was different for the test compounds and differences could be rationalised on the basis of the administration route and physico-chemical properties of the administered compound and its metabolites. In another study, the origin of the main DNA adduct found in tissues of rats fed with raw broccoli could be related to the glucosinolate neoglucobrassicin [35,36].

Roel Vermeulen (Utrecht University, The Netherlands) discussed 'The use of intermediate endpoint biomarkers in cancer aetiology and risk assessment'. Intermediate biomarkers directly or indirectly represent events on the continuum between exposure and disease. As such, they complement epidemiological studies that use cancer endpoints. In addition, intermediate biomarkers can provide initial clues about the carcinogenic potential of new exposures before cancer develops. Benzene was presented as an example [37,38] for which intermediate markers are more frequently being used in cancer risk assessment [39].

The second keynote lecture was given by Steffen Loft (University of Copenhagen, Denmark) on 'Oxidative damage to DNA as biomarker of exposure to air pollution'. Air pollution is suspected of causing lung cancer [40] and traffic is one of the major sources of harmful airborne particles worldwide [41]. Air pollution is thought to exert health effects through oxidative stress which includes the formation of DNA damage [42]. Individual assessment of exposure to combustion-derived particulate matter may be improved by measuring biomarkers of oxidative damage to DNA in humans. For DNA lesions in leukocytes it is recommended to use methods such as the FPG- or hOGG1-modified comet assay, whereas for urinary detection of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) chromatographic assays with specific detection by mass spectrometry or electrochemistry are preferred. Series of studies showed that exposure to traffic-related combustion particles increased DNA oxidation in leukocytes, whereas wood smoke particles appeared to have less effect. Exposure to combustion particles consistently increases the level of oxidatively

damaged DNA in humans supporting the use of measurements as biomarkers of biological effective dose. Peter Farmer (University of Leicester, UK) reported on ‘The role of oxidative DNA damage markers in molecular cancer epidemiology studies: 8-oxodG – is there an alternative?’ The most frequently used biomarker of DNA damage linked to oxidative stress is the amount of 8-oxodG present in DNA [43,44]. However, this lesion may be produced spuriously during DNA isolation and work-up procedures. Another approach to indicate exposure to reactive oxygen species (ROS) is the analysis of the DNA adduct with malondialdehyde, 3-(2'-deoxy- $\beta$ -D-erythro-pentofuranosyl)pyrimido[1,2- $\alpha$ ]purin-10(3H)-one (M<sub>1</sub>dG), as M<sub>1</sub>dG is not subject to artifactual formation [43,44]. An immunoslot blot procedure for determination of M<sub>1</sub>dG was developed and M<sub>1</sub>dG levels were influenced by exposure to air pollution and by dietary influences, suggesting its potential as an oxidative DNA damage biomarker. Another potential biomarker of oxidative stress may be 7-(2-hydroxyethyl)guanine, a DNA adduct produced by ethylene oxide [45]. Marcus Cooke (University of Leicester, UK) discussed the measurement of 8-oxodG in urine [46] using both ELISA and chromatographic methods [47]. The European Standards Committee on Urinary (DNA) Lesion Analysis (ESCUA) [48] comprises over 30 laboratories from Europe, USA and Japan to address questions of lesion provenance and methodological variation, with a view to providing analyses that are highly applicable to large, population-based studies of genotoxic exposure and, with appropriate validation, to clinical studies.

Andrew Collins (University of Oslo, Norway) discussed ‘Antioxidant and non-antioxidant effects of phytochemicals’. The comet assay has proved to be particularly useful as a human biomonitoring tool. The use of enzymes that convert oxidised bases to DNA breaks has made it possible to measure oxidative damage specifically, and to assess antioxidant protection against this damage [49]. Although it has been possible to detect the effects of natural antioxidants (*e.g.* vitamin C,  $\beta$ -carotene, flavonoids) on the antioxidant status, large-scale intervention trials showed that antioxidants per se do not protect against cancer. Still, it is evident that fruits and vegetables are protective against cancer, suggesting that other pathways (*e.g.* DNA repair) [50] may be influenced by phytochemicals, in addition to their action as antioxidants. Both cell culture experiments and human intervention trials have shown an influence of phytochemicals on DNA repair and it appears that effects on different repair pathways are variable. Roger Godschalk (University of Maastricht, The Netherlands) reported on ‘Biomarkers of DNA damage and repair in nutritional studies’. In order to investigate the mechanism behind the procarcinogenic effect of  $\beta$ -carotene in smokers’ lung cancer, the effects of  $\beta$ -carotene and its metabolites vitamin A and retinoic acid

on neutrophil-induced genotoxicity were examined [51].  $\beta$ -carotene metabolites enhanced inflammation-induced oxidative stress and genetic damage [51], and oxidative stress can reduce the DNA repair (*i.e.* nucleotide excision repair [NER]) capacity [52]. The effect of dietary intake of antioxidants on an individual's NER capacity was measured in lymphocytes of individuals ( $n=168$ ) before and after a 4-week dietary intervention of blueberry and apple juice [53]. On average the intervention did not affect NER capacity, but the effects may be modulated by genetic polymorphisms (*e.g.* XPA G23A) in NER-related genes. Karen Brown (University of Leicester, UK) delivered a talk on 'Resveratrol: from glass to bedside'. Clinical trials of tamoxifen and aspirin have provided proof that cancer prevention in humans is feasible [54]. Therefore, chemicals derived from the diet such as resveratrol found in red wine have been tested in healthy volunteers and in cancer patients in Leicester [55]. Comprehensive clinical trials assessing resveratrol pharmacokinetics have revealed that despite propensity for rapid metabolism, the parent compound predominates in colon tissue, supporting the colorectum as a target [56]. In contrast, resveratrol itself was not detectable in human prostate after a single dose, indicating efficacy in internal organs may be dependent on the potential contribution of metabolites. Results suggested a non-linear dose-response for the protective effect of resveratrol against colon cancer suggesting that lower doses of resveratrol may be more efficacious. These findings illustrate the need to integrate putative efficacy biomarkers early in the development process to identify optimal doses for clinical evaluation.

Roel Schins (IUF-Leibnitz Research Institute for Environmental Medicine, Germany) discussed 'Particles, inflammation and oxidative DNA damage'. Particle-induced ROS formation in lung epithelial cells is implicated in the activation of signalling pathways that drive pulmonary inflammation; marked increased in ROS formation may also cause oxidative damage to DNA [57]. For particle risk assessment it is important to discriminate between oxidative damage to DNA that results from ROS (*i*) generated in lung epithelial cells upon interaction with the particles or (*ii*) by activated phagocytes during lung inflammation. This is also important for newly engineered nanoparticles (ENPs) for which, besides the lung, the gastrointestinal tract represents the major target organ [58]. It was shown that several ENPs can cause oxidative damage to DNA in human Caco-2 intestinal epithelial cells [59]. Activated neutrophils also induced oxidative damage to DNA in Caco-2 cells focusing future studies on the interaction between ENPs and phagocytic cells during inflammation. Hanna Karlsson (Karolinska Institutet, Sweden) delivered a talk on 'Nanoparticles and oxidative DNA damage – Trojan horses and assay interactions'. By using the comet assay it was shown that copper oxide (CuO) nanoparticles were most potent in causing DNA damage and

oxidative lesions in DNA when comparing different metal oxide nanoparticles [60]. Higher toxicity as well as higher intracellular concentrations of copper were observed after exposure to the particles compared to the ions. Thus it seems that CuO nanoparticles act via a Trojan horse type mechanism, *i.e.* the particle structure increases cellular uptake and once inside the cell toxicity is likely caused by a combination of metal ions and a reactive surface of the CuO nanoparticles [61]. It was also noted that, when assessing DNA damage caused by nanoparticles using the comet assay, particles are often visible in the comet head which may lead to additional damage during the assay performance and/or interactions with the FPG enzymes used for the detection of oxidative damage to DNA [62]. Tanja Schwerdtle (University of Münster, Germany) reported on 'Exposure relevant arsenic and manganese species strongly inhibit cellular oxidative DNA damage response pathways'. At exposure-relevant concentrations the human carcinogen inorganic arsenic and its methylated metabolites are neither directly DNA reactive nor mutagenic. Therefore more likely epigenetic and indirect genotoxic effects, among others a modulation of DNA repair pathways [63,64], are important molecular mechanisms contributing to arsenic carcinogenicity. Thus in cultured human lung cells arsenic species affected base excision repair (BER) of oxidative damage to DNA by several mechanisms. Therefore after mixed arsenic exposure in humans, DNA repair most likely will be affected by different mechanisms. Regarding the mode of neurotoxic action of manganese new studies provide evidence that after overload manganese strongly disturbs the blood-brain barrier and the blood-cerebrospinal fluid (CSF) barrier and then might disturb DNA damage response in brain cells [65], which has been shown before to result in neurological diseases.

Bernd Epe (University of Mainz, Germany) discussed 'Basal levels of oxidative DNA modifications in mice: origin, relevance and modulation'. A small increase of the basal level of oxidative DNA base modifications (*i.e.* less than one additional 8-oxodG per  $10^6$  base pairs) gives rise to a doubling of the spontaneous mutation rate and initiate carcinogenesis [66]. This was demonstrated in mice deficient in OGG1, the major repair glycosylase for 8-oxodG. Oral dosing of mice with resveratrol significantly reduced the basal levels of endogenous oxidative DNA base modifications in the livers of both WT and repair-deficient animals [67]. The protection appears to be mediated by an up-regulation of several antioxidant response proteins (*e.g.* SOD1, SOD2, GPX1, HO1) which was observed in hepatocytes of resveratrol-treated animals. Oxidative stress not only increased the generation of oxidative damage to DNA, but also affected OGG1-mediated repair of the lesions. The human Ogg1-C326 variant appeared to be particularly sensitive to oxidative stress, resulting

in repair retardation and elevated genetic instability [68]. Eugenia Dogliotti (Istituto Superiore di Sanità, Italy) reported on ‘Genetic susceptibility to oxidative damage: from SNPs to function’. A genotype-phenotype correlation study was carried out in 225 healthy volunteers to identify *OGG1* polymorphisms associated with impaired functional activity or altered gene expression. OGG1 activity was measured in protein extracts prepared from peripheral lymphocytes by using a fluorescence-based *in vitro* cleavage assay [69]. A significantly lower activity was detected in extracts from subjects homozygous for the C326 allele when compared with those homozygous for the S326 allele. A weakly significant inverse correlation was found between cleavage activity and gene expression but no association was found between *OGG1* gene expression and the S326C polymorphism. The loss of enzymatic activity of the S326 polymorphic variant protein could be a consequence of conformational changes. These data confirm that the redox environment is a determinant of the repair efficiency of OGG1 and the susceptibility to oxidative stress of the S326C variant may account for its association with increased cancer risk. Ryszard Olinski (Nicolaus Copernicus University, Poland) discussed ‘Oxidatively damaged DNA in *BRCA1* mutations carriers’ [70]. The study included healthy individuals, carriers of the *BRCA1* mutation without symptoms of disease and patients with breast and ovarian cancer with *BRCA1* mutations. *BRCA1* mutation carriers had elevated levels of 8-oxodG in DNA of lymphocytes. In the distinct subpopulation of *BRCA1* mutation carriers without symptoms of cancer who underwent adnexectomy, supplementation with selenium significantly decreased the level of 8-oxodG in comparison with the subgroup without supplementation [71]. Selenium supplementation probably leads to an increase in BER enzyme activity (*i.e.* OGG1 activity) which in turn may result in the reduction of oxidative damage to DNA.

Helga Stopper delivered a talk on ‘Oxidative DNA damage during aging in a *p47phox* knock-out model’. NADPH oxidase is one enzyme mediating cellular production of ROS. Oxidative stress markers and genomic damage were evaluated in *p47phox*( $-/-$ ) mice, a knock-out model with a deletion of the p47phox subunit of NADH oxidase. Oxidative stress markers including 8-oxodG were significantly higher in urine of young (8-10 weeks) and old (52 weeks) WT mice than *p47phox*( $-/-$ ) mice. The levels of 8-oxodG from kidney DNA were also significantly different in WT and *p47phox*( $-/-$ ) mice in both young and old animals, with adduct levels increasing age-dependently. Treatment of isolated kidney cells with angiotensin II, shown to cause NADPH oxidase-mediated oxidative damage to DNA [72], resulted in significantly higher level of DNA damage in WT cells of both age groups, but no difference was found for cells isolated from *p47phox*( $-/-$ ) mice. Collectively, the p47phox subunit of

NADPH oxidase plays an important role in the formation of 8-oxodG. This represents a mechanism how NADPH oxidase is involved in the formation of ROS-mediated DNA damage which accumulates during aging.

Thanks to Mutagenesis and Oxford University Press three poster awards were given to young scientists: 1) Kathrin Gallé (University of Mainz, Germany) – ‘Overexpression of human Cu/Zn superoxide dismutase prevents the accumulation of oxidative DNA base damage in *Ogg1<sup>-/-</sup>Csb<sup>-/-</sup>* double knockout mice’; 2) Kristin Herrman (German Institute of Human Nutrition, Germany) – ‘Mass spectrometric detection and quantification of DNA adducts: usage for the identification of human and murine SULT forms involved in the activation of methyleugenol *in vitro* and in animal models’; and 3) Alicia Pains (Wageningen University, The Netherlands) – ‘The levels of estragole DNA adducts formed *in vivo* corresponds to the levels predicted by a physiologically based biodynamic (PBBD) model established *in vitro*’. All oral and poster abstracts of the workshop have been published online in Mutagenesis [\(include URL here\)](#).

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## References

1. Wild, C.P. (2009) Environmental exposure measurement in cancer epidemiology. *Mutagenesis*, **24**, 117-25.
2. Wild, C.P. (2011) Future research perspectives on environment and health: the requirement for a more expansive concept of translational cancer research. *Environ Health*, **10 Suppl 1**, S15.
3. Arlt, V.M., Frei, E. and Schmeiser, H.H. (2007) ECNIS-sponsored workshop on biomarkers of exposure and cancer risk: DNA damage and DNA adduct detection and 6th GUM-32P-postlabelling workshop, German Cancer Research Center, Heidelberg, Germany, 29-30 September 2006. *Mutagenesis*, **22**, 83-8.
4. Hecht, S.S., Yuan, J.M. and Hatsukami, D. (2010) Applying tobacco carcinogen and toxicant biomarkers in product regulation and cancer prevention. *Chem Res Toxicol*, **23**, 1001-8.
5. Thomas, J.L., Guo, H., Carmella, S.G., Balbo, S., Han, S., Davis, A.B., Yoder, A.R., Murphy, S.E., An, L.C., Ahluwalia, J.S. and Hecht, S.S. (2011) Metabolites of a Tobacco-Specific Lung Carcinogen in Children Exposed to Secondhand or Thirdhand Tobacco Smoke in Their Homes. *Cancer Epidemiol Biomarkers Prev*.
6. Yaun, J.M., Gao, Y.T., Carmella, S.G., Wang, R., Zhong, Y., Tao, L., Davis, A.B., Moy, K.A., Chen, M., Han, S., Yu, M.C. and Hecht, S.S. (2010) Urinary levels of polycyclic aromatic hydrocarbon metabolite in relation to lung cancer development in cigarette smokers of the Shanghai Cohort Study. *In: Proceedings of the 101st Annual Meeting of the American Association for Cancer Research; 2010 Apr 17-21; Washington, DC. Philadelphia (PA) USA* A863.
7. Seidel, A., Spickenheuer, A., Straif, K., Rihs, H.P., Marczynski, B., Scherenberg, M., Dettbarn, G., Angerer, J., Wilhelm, M., Bruning, T., Jacob, J. and Pesch, B. (2008) New biomarkers of occupational exposure to polycyclic aromatic hydrocarbons. *J Toxicol Environ Health A*, **71**, 734-45.
8. Marczynski, B., Pesch, B., Wilhelm, M., Rossbach, B., Preuss, R., Hahn, J.U., Rabstein, S., Raulf-Heimsoth, M., Seidel, A., Rihs, H.P., Adams, A., Scherenberg, M., Erkes, A., Engelhardt, B., Straif, K., Kafferlein, H.U., Angerer, J. and Bruning, T. (2009) Occupational exposure to polycyclic aromatic hydrocarbons and DNA damage by industry: a nationwide study in Germany. *Arch Toxicol*, **83**, 947-57.
9. De Flora, S. and Izzotti, A. (2009) Modulation of genomic and postgenomic alterations in noncancer diseases and critical periods of life. *Mutat Res*, **667**, 15-26.
10. Balansky, R., Ganchev, G., Iltcheva, M., Steele, V.E. and De Flora, S. (2010) Prevention of cigarette smoke-induced lung tumors in mice by budesonide, phenethyl isothiocyanate, and N-acetylcysteine. *Int J Cancer*, **126**, 1047-54.
11. Balansky, R., Ganchev, G., Iltcheva, M., Nikolov, M., Steele, V.E. and De Flora, S. (2011) Differential carcinogenicity of cigarette smoke in mice exposed either transplacentally, early in life or in adulthood. *Int J Cancer*.
12. John, K., Divi, R.L., Keshava, C., Orozco, C.C., Schockley, M.E., Richardson, D.L., Poirier, M.C., Nath, J. and Weston, A. (2010) CYP1A1 and CYP1B1 gene expression and DNA adduct formation in normal human mammary epithelial cells exposed to benzo[a]pyrene in the absence or presence of chlorophyllin. *Cancer Lett*, **292**, 254-60.
13. van Gijssel, H.E., Schild, L.J., Watt, D.L., Roth, M.J., Wang, G.Q., Dawsey, S.M., Albert, P.S., Qiao, Y.L., Taylor, P.R., Dong, Z.W. and Poirier, M.C. (2004) Polycyclic aromatic hydrocarbon-DNA adducts determined by semiquantitative immunohistochemistry in human esophageal biopsies taken in 1985. *Mutat Res*, **547**, 55-62.
14. Arlt, V.M., Stiborova, M., Henderson, C.J., Thiemann, M., Frei, E., Aimova, D., Singh, R., Gamboa da Costa, G., Schmitz, O.J., Farmer, P.B., Wolf, C.R. and Phillips,



- D.H. (2008) Metabolic activation of benzo[a]pyrene in vitro by hepatic cytochrome P450 contrasts with detoxification in vivo: experiments with hepatic cytochrome P450 reductase null mice. *Carcinogenesis*, **29**, 656-65.
15. Hockley, S.L., Arlt, V.M., Jahnke, G., Hartwig, A., Giddings, I. and Phillips, D.H. (2008) Identification through microarray gene expression analysis of cellular responses to benzo(a)pyrene and its diol-epoxide that are dependent or independent of p53. *Carcinogenesis*, **29**, 202-10.
  16. Van Berkel, J.J., Dallinga, J.W., Moller, G.M., Godschalk, R.W., Moonen, E.J., Wouters, E.F. and Van Schooten, F.J. (2010) A profile of volatile organic compounds in breath discriminates COPD patients from controls. *Respir Med*, **104**, 557-63.
  17. Schults, M.A., Timmermans, L., Godschalk, R.W., Theys, J., Wouters, B.G., van Schooten, F.J. and Chiu, R.K. (2010) Diminished carcinogen detoxification is a novel mechanism for hypoxia-inducible factor 1-mediated genetic instability. *J Biol Chem*, **285**, 14558-64.
  18. Decordier, I., Papine, A., Plas, G., Roesems, S., Vande Loock, K., Moreno-Palomo, J., Cemeli, E., Anderson, D., Fucic, A., Marcos, R., Soussaline, F. and Kirsch-Volders, M. (2009) Automated image analysis of cytokinesis-blocked micronuclei: an adapted protocol and a validated scoring procedure for biomonitoring. *Mutagenesis*, **24**, 85-93.
  19. Decordier, I., De Bont, K., De Bock, K., Mateuca, R., Roelants, M., Ciardelli, R., Haumont, D., Knudsen, L.E. and Kirsch-Volders, M. (2007) Genetic susceptibility of newborn daughters to oxidative stress. *Toxicol Lett*, **172**, 68-84.
  20. Decordier, I., Loock, K.V. and Kirsch-Volders, M. (2010) Phenotyping for DNA repair capacity. *Mutat Res*, **705**, 107-29.
  21. Speit, G., Zeller, J. and Neuss, S. (2011) The in vivo or ex vivo origin of micronuclei measured in human biomonitoring studies. *Mutagenesis*, **26**, 107-10.
  22. Georgiadis, P., Kaila, S., Makedonopoulou, P., Fthenou, E., Chatzi, L., Pletsa, V. and Kyrtopoulos, S.A. (2011) Development and validation of a new, sensitive immunochemical assay for O-methylguanine in DNA and its application in a population study. *Cancer Epidemiol Biomarkers Prev*, **20**, 82-90.
  23. Merlo, D.F., Wild, C.P., Kogevinas, M., Kyrtopoulos, S. and Kleinjans, J. (2009) NewGeneris: a European study on maternal diet during pregnancy and child health. *Cancer Epidemiol Biomarkers Prev*, **18**, 5-10.
  24. Funk, D., Sorg, B.L., Lindner, S.C. and Schmeiser, H.H. (2010) 32P-postlabeling analysis of DNA adducts formed by leukotriene A4 (LTA4). *Environ Mol Mutagen*, **51**, 338-43.
  25. Topinka, J., Rossner, P., Jr., Milcova, A., Schmuczerova, J., Svecova, V. and Sram, R.J. (2011) DNA adducts and oxidative DNA damage induced by organic extracts from PM2.5 in an acellular assay. *Toxicol Lett*, **202**, 186-92.
  26. Dracinsky, M., Cvacka, J., Semanska, M., Martinek, V., Frei, E. and Stiborova, M. (2009) Mechanism of formation of (deoxy)guanosine adducts derived from peroxidase-catalyzed oxidation of the carcinogenic nonaminoazo dye 1-phenylazo-2-hydroxynaphthalene (Sudan I). *Chem Res Toxicol*, **22**, 1765-73.
  27. Naiman, K., Dracinska, H., Martinkova, M., Sulc, M., Dracinsky, M., Kejikova, L., Hodek, P., Hudecek, J., Liberda, J., Schmeiser, H.H., Frei, E. and Stiborova, M. (2008) Redox cycling in the metabolism of the environmental pollutant and suspected human carcinogen o-anisidine by rat and rabbit hepatic microsomes. *Chem Res Toxicol*, **21**, 1610-21.
  28. Mizerovska, J., Dracinska, H., Frei, E., Schmeiser, H.H., Arlt, V.M. and Stiborova, M. (2011) Induction of biotransformation enzymes by the carcinogenic air-pollutant 3-nitrobenzanthrone in liver, kidney and lung, after intra-tracheal instillation in rats. *Mutat Res*, **720**, 34-41.

29. Stiborova, M., Martinek, V., Svobodova, M., Sistkova, J., Dvorak, Z., Ulrichova, J., Simanek, V., Frei, E., Schmeiser, H.H., Phillips, D.H. and Arlt, V.M. (2010) Mechanisms of the different DNA adduct forming potentials of the urban air pollutants 2-nitrobenzanthrone and carcinogenic 3-nitrobenzanthrone. *Chem Res Toxicol*, **23**, 1192-201.
30. Schmeiser, H.H., Stiborova, M. and Arlt, V.M. (2009) Chemical and molecular basis of the carcinogenicity of Aristolochia plants. *Curr Opin Drug Discov Devel*, **12**, 141-8.
31. Kucab, J.E., Phillips, D.H. and Arlt, V.M. (2010) Linking environmental carcinogen exposure to TP53 mutations in human tumours using the human TP53 knock-in (Hupki) mouse model. *Febs J*, **277**, 2567-83.
32. Levova, K., Moserova, M., Kotrbova, V., Sulc, M., Henderson, C.J., Wolf, C.R., Phillips, D.H., Frei, E., Schmeiser, H.H., Mares, J., Arlt, V.M. and Stiborova, M. (2011) Role of Cytochromes P450 1A1/2 in Detoxication and Activation of Carcinogenic Aristolochic Acid I: Studies with the Hepatic NADPH: Cytochrome P450 Reductase Null (HRN) Mouse Model. *Toxicol Sci*, **121**, 43-56.
33. Doerge, D.R., da Costa, G.G., McDaniel, L.P., Churchwell, M.I., Twaddle, N.C. and Beland, F.A. (2005) DNA adducts derived from administration of acrylamide and glycidamide to mice and rats. *Mutat Res*, **580**, 131-41.
34. Von Tungeln, L.S., Churchwell, M.I., Doerge, D.R., Shaddock, J.G., McGarrity, L.J., Heflich, R.H., da Costa, G.G., Marques, M.M. and Beland, F.A. (2009) DNA adduct formation and induction of micronuclei and mutations in B6C3F1/Tk mice treated neonatally with acrylamide or glycidamide. *Int J Cancer*, **124**, 2006-15.
35. Baasanjav-Gerber, C., Hollnagel, H.M., Brauchmann, J., Iori, R. and Glatt, H. (2010) Detection of genotoxicants in Brassicales using endogenous DNA as a surrogate target and adducts determined by 32P-postlabelling as an experimental end point. *Mutagenesis*.
36. Baasanjav-Gerber, C., Hollnagel, H.M., Brauchmann, J., Iori, R. and Glatt, H. (2011) Detection of genotoxicants in Brassicales using endogenous DNA as a surrogate target and adducts determined by 32P-postlabelling as an experimental end point. *Mutagenesis*, **26**, 407-13.
37. Lan, Q., Zhang, L., Li, G., Vermeulen, R., Weinberg, R.S., Dosemeci, M., Rappaport, S.M., Shen, M., Alter, B.P., Wu, Y., Kopp, W., Waidyanatha, S., Rabkin, C., Guo, W., Chanock, S., Hayes, R.B., Linet, M., Kim, S., Yin, S., Rothman, N. and Smith, M.T. (2004) Hematotoxicity in workers exposed to low levels of benzene. *Science*, **306**, 1774-6.
38. Vlaanderen, J., Lan, Q., Kromhout, H., Rothman, N. and Vermeulen, R. (2011) Occupational benzene exposure and the risk of lymphoma subtypes: a meta-analysis of cohort studies incorporating three study quality dimensions. *Environ Health Perspect*, **119**, 159-67.
39. Baan, R., Grosse, Y., Straif, K., Secretan, B., El Ghissassi, F., Bouvard, V., Benbrahim-Tallaa, L., Guha, N., Freeman, C., Galichet, L. and Coglian, V. (2009) A review of human carcinogens--Part F: chemical agents and related occupations. *Lancet Oncol*, **10**, 1143-4.
40. Raaschou-Nielsen, O., Bak, H., Sorensen, M., Jensen, S.S., Ketznel, M., Hvidberg, M., Schnohr, P., Tjonneland, A., Overvad, K. and Loft, S. (2010) Air pollution from traffic and risk for lung cancer in three Danish cohorts. *Cancer Epidemiol Biomarkers Prev*, **19**, 1284-91.
41. Londahl, J., Massling, A., Swietlicki, E., Brauner, E.V., Ketznel, M., Pagels, J. and Loft, S. (2009) Experimentally determined human respiratory tract deposition of airborne particles at a busy street. *Environ Sci Technol*, **43**, 4659-64.

42. Moller, P. and Loft, S. (2010) Oxidative damage to DNA and lipids as biomarkers of exposure to air pollution. *Environ Health Perspect*, **118**, 1126-36.
43. Singh, R., Kaur, B., Kalina, I., Popov, T.A., Georgieva, T., Garte, S., Binkova, B., Sram, R.J., Taioli, E. and Farmer, P.B. (2007) Effects of environmental air pollution on endogenous oxidative DNA damage in humans. *Mutat Res*, **620**, 71-82.
44. Singh, R., Sram, R.J., Binkova, B., Kalina, I., Popov, T.A., Georgieva, T., Garte, S., Taioli, E. and Farmer, P.B. (2007) The relationship between biomarkers of oxidative DNA damage, polycyclic aromatic hydrocarbon DNA adducts, antioxidant status and genetic susceptibility following exposure to environmental air pollution in humans. *Mutat Res*, **620**, 83-92.
45. Marsden, D.A., Jones, D.J., Britton, R.G., Ognibene, T., Ubick, E., Johnson, G.E., Farmer, P.B. and Brown, K. (2009) Dose-response relationships for N7-(2-hydroxyethyl)guanine induced by low-dose [14C]ethylene oxide: evidence for a novel mechanism of endogenous adduct formation. *Cancer Res*, **69**, 3052-9.
46. Evans, M.D., Saporbaev, M. and Cooke, M.S. (2010) DNA repair and the origins of urinary oxidized 2'-deoxyribonucleosides. *Mutagenesis*, **25**, 433-42.
47. Evans, M.D., Singh, R., Mistry, V., Sandhu, K., Farmer, P.B. and Cooke, M.S. (2008) Analysis of urinary 8-oxo-7,8-dihydro-purine-2'-deoxyribonucleosides by LC-MS/MS and improved ELISA. *Free Radic Res*, **42**, 831-40.
48. Evans, M.D., Olinski, R., Loft, S. and Cooke, M.S. (2010) Toward consensus in the analysis of urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine as a noninvasive biomarker of oxidative stress. *FASEB J*, **24**, 1249-60.
49. Collins, A.R. and Azqueta, A. (2011) DNA repair as a biomarker in human biomonitoring studies; further applications of the comet assay. *Mutat Res*.
50. Ramos, A.A., Pereira-Wilson, C. and Collins, A.R. (2010) Protective effects of ursolic acid and luteolin against oxidative DNA damage include enhancement of DNA repair in Caco-2 cells. *Mutat Res*, **692**, 6-11.
51. van Helden, Y.G., Keijer, J., Knaapen, A.M., Heil, S.G., Briede, J.J., van Schooten, F.J. and Godschalk, R.W. (2009) Beta-carotene metabolites enhance inflammation-induced oxidative DNA damage in lung epithelial cells. *Free Radic Biol Med*, **46**, 299-304.
52. Gungor, N., Godschalk, R.W., Pachon, D.M., Van Schooten, F.J. and Knaapen, A.M. (2007) Activated neutrophils inhibit nucleotide excision repair in human pulmonary epithelial cells: role of myeloperoxidase. *FASEB J*, **21**, 2359-67.
53. Langie, S.A., Wilms, L.C., Hamalainen, S., Kleinjans, J.C., Godschalk, R.W. and van Schooten, F.J. (2010) Modulation of nucleotide excision repair in human lymphocytes by genetic and dietary factors. *Br J Nutr*, **103**, 490-501.
54. Patel, K.R., Scott, E., Brown, V.A., Gescher, A.J., Steward, W.P. and Brown, K. (2011) Clinical trials of resveratrol. *Ann N Y Acad Sci*, **1215**, 161-9.
55. Brown, V.A., Patel, K.R., Viskaduraki, M., Crowell, J.A., Perloff, M., Booth, T.D., Vasilinin, G., Sen, A., Schinas, A.M., Piccirilli, G., Brown, K., Steward, W.P., Gescher, A.J. and Brenner, D.E. (2010) Repeat dose study of the cancer chemopreventive agent resveratrol in healthy volunteers: safety, pharmacokinetics, and effect on the insulin-like growth factor axis. *Cancer Res*, **70**, 9003-11.
56. Patel, K.R., Brown, V.A., Jones, D.J., Britton, R.G., Hemingway, D., Miller, A.S., West, K.P., Booth, T.D., Perloff, M., Crowell, J.A., Brenner, D.E., Steward, W.P., Gescher, A.J. and Brown, K. (2010) Clinical pharmacology of resveratrol and its metabolites in colorectal cancer patients. *Cancer Res*, **70**, 7392-9.
57. Schins, R.P. and Knaapen, A.M. (2007) Genotoxicity of poorly soluble particles. *Inhal Toxicol*, **19 Suppl 1**, 189-98.

58. Donaldson, K., Poland, C.A. and Schins, R.P. (2010) Possible genotoxic mechanisms of nanoparticles: criteria for improved test strategies. *Nanotoxicology*, **4**, 414-20.
59. Gerloff, K., Albrecht, C., Boots, A.W., Foerster, I. and Schins, R.P.F. (2009) Cytotoxicity and oxidative DNA damage by nanoparticles in human intestinal Caco-2 cells. *Nanotoxicology*, **3**, 355-364.
60. Karlsson, H.L., Cronholm, P., Gustafsson, J. and Moller, L. (2008) Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes. *Chem Res Toxicol*, **21**, 1726-32.
61. Karlsson, H.L., Holgersson, A. and Moller, L. (2008) Mechanisms related to the genotoxicity of particles in the subway and from other sources. *Chem Res Toxicol*, **21**, 726-31.
62. Karlsson, H.L. (2010) The comet assay in nanotoxicology research. *Anal Bioanal Chem*, **398**, 651-66.
63. Hartwig, A. and Schwerdtle, T. (2009) Arsenic induced carcinogenicity: new insights in molecular mechanisms. In Hadjiladis, N. and Sletten, E. (eds.), *Metal-Complex DNA Interactions*. John Wiley & Sons, pp. 491-582.
64. Nollen, M., Ebert, F., Moser, J., Mullenders, L.H., Hartwig, A. and Schwerdtle, T. (2009) Impact of arsenic on nucleotide excision repair: XPC function, protein level, and gene expression. *Mol Nutr Food Res*, **53**, 572-82.
65. Bornhorst, J., Ebert, F., Hartwig, A., Michalke, B. and Schwerdtle, T. (2010) Manganese inhibits poly(ADP-ribosylation) in human cells: a possible mechanism behind manganese-induced toxicity? *J Environ Monit*, **12**, 2062-9.
66. Trapp, C., Reite, K., Klungland, A. and Epe, B. (2007) Deficiency of the Cockayne syndrome B (CSB) gene aggravates the genomic instability caused by endogenous oxidative DNA base damage in mice. *Oncogene*, **26**, 4044-8.
67. Fusser, M., Nesse, G.J., Khobta, A., Xia, N., Li, H., Klungland, A. and Epe, B. (2011) Spontaneous mutagenesis in Csb(m/m)Ogg1(/) mice is attenuated by dietary resveratrol. *Carcinogenesis*, **32**, 80-5.
68. Bravard, A., Vacher, M., Moritz, E., Vaslin, L., Hall, J., Epe, B. and Radicella, J.P. (2009) Oxidation status of human OGG1-S326C polymorphic variant determines cellular DNA repair capacity. *Cancer Res*, **69**, 3642-9.
69. Mazzei, F., Guarrera, S., Allione, A., Simonelli, V., Narciso, L., Barone, F., Minoprio, A., Ricceri, F., Funaro, A., D'Errico, M., Vogel, U., Matullo, G. and Dogliotti, E. (2011) 8-Oxoguanine DNA-glycosylase repair activity and expression: a comparison between cryopreserved isolated lymphocytes and EBV-derived lymphoblastoid cell lines. *Mutat Res*, **718**, 62-7.
70. Dziaman, T., Huzarski, T., Gackowski, D., Rozalski, R., Siomek, A., Szpila, A., Guz, J., Lubinski, J. and Olinski, R. (2009) Elevated level of 8-oxo-7,8-dihydro-2'-deoxyguanosine in leukocytes of BRCA1 mutation carriers compared to healthy controls. *Int J Cancer*, **125**, 2209-13.
71. Dziaman, T., Huzarski, T., Gackowski, D., Rozalski, R., Siomek, A., Szpila, A., Guz, J., Lubinski, J., Wasowicz, W., Roszkowski, K. and Olinski, R. (2009) Selenium supplementation reduced oxidative DNA damage in adnexectomized BRCA1 mutations carriers. *Cancer Epidemiol Biomarkers Prev*, **18**, 2923-8.
72. Schmid, U., Stopper, H., Schweda, F., Queisser, N. and Schupp, N. (2008) Angiotensin II induces DNA damage in the kidney. *Cancer Res*, **68**, 9239-46.